*Warsaw Agricultural University, Warsaw, Poland
** Research Institute of Pomology and Floriculture, Skierniewice, Poland

DIFFERENCES IN ELECTROPHORETIC MOBILITY OF COAT PROTEIN AMONG 13 *PRUNUS NECROTIC RINGSPOT VIRUS* (PNRSV) ISOLATES

*E. Paduch-Cichal, *K. Sala-Rejczak and **T. Malinowski

Abstract

The electrophoretic mobility of the coat protein was compared for 13 PNRSV isolates. Apparent molecular weight varied from about 24 to 25 kDa. The molecular weight of coat protein of two isolates: PNRSV-AprI/9 and PNRSV-PL21 could not be determined.

Key words: PNRSV, virus isolates, western blotting technique

Introduction

Prunus necrotic ringspot virus (PNRSV) belongs to the genus *Ilarvirus* of the family *Bromoviridae* (Roossinck et al. 2005).

The molecular weight (Mγ) of coat protein of different PNRSV isolates were reported by many researchers and they varied among virus isolates. Barnett and Fulton (1969) reported a Mγ of 25 kDa for PNRSV isolate H based on amino acids analysis. Gonsalves and Fulton (1977) determined a Mγ of 25 kDa for PNRSV isolate G. Ong (1987) noted variation in Mγ among three PNRSV isolates: 26 kDa for FG (isolate from *Prunus mahaleb*) and CH39 (isolate from *P. avium*), and 26.5 kDa for CH38 (isolate from *P. avium*). Crosslin and Mink (1992) and Paduch-Cichal (2000) demonstrated that the molecular weight of PNRSV coat protein varied among almond, peach, sour cherry, sweet cherry, hop and rose isolates from 22 to 29 kDa. The viral capsid protein of seven Slovak PNRSV isolates (plum, sweet and sour cherry) migrated in SDS-PAGE as one major band with apparent molecular weight of about 25 kDa (Glasa et al. 2000).

Phytopathol. Pol. 44: 37–42

ISSN 1230-0462

This paper reports the results of a study carried out to compare the electrophoretic mobility of coat protein of 15 PNRSV isolates from different stone fruit trees and rose plants.

Material and methods

The virus isolates used in this study are listed in Table 1.

Samples (leaf breaking buds) from *P. avium* F12/1 infected with PNRSV-AL1, PNRSV-AL17, PNRSV-AprI/9, PNRSV-N2, PNRSV-Mk, PNRSV-PE56, PNRSV-PL1, PNRSV-PL9, PNRSV-PL38, PNRSV-SW2, PNRSV-R1, PNRSV-R2 or PNRSV-R3 and samples from plum cv. 'Empress' (leaf breaking buds) infected with PNRSV-PL7 or PNRSV-PL21 were prepared for western blotting by grinding with sample buffer (0.5 M Tris-HCl buffer, pH 6.8, containing 10% glycerol, 10% SDS, 2-mercaptoethanol, 0.05% bromophenol blue) and centrifuged for 15 min at 15 000 rpm (centrifuge Janetzky, K24). The supernatant was incubated for 5 min at 95°C (Sherwood and Melouk 1986, Sherwood 1987).

The samples were subjected to electrophoresis in 12% polyacrylamide running gel with a 4% stacking gel (Laemmli 1970). Low molecular weight markers (Sigma) were run in parallel. After electrophoresis, all proteins were transferred onto PVDF membrane (Millipore) in a semi-dry apparatus (Pharmacia). PVDF membranes were blocked with 3% semi-skimmed milk in TBS buffer, incubated with a mixture of rabbit antisera against PNRSV (S PNRSV 97-4 and S PNRSV 11) in TBS for 4 h at room temperature (Towbin et al. 1979), followed by Protein A / ALP conjugate (Sigma) and Fast Red RC / Naphtol AS-TR (Sigma).

The molecular weight (Mγ) of coat proteins of PNRSV isolates was estimated by comparison with Sigma Low Molecular Weight Range markers (M-3813). Those were stained on PVDF with Coomassie Blue stain.

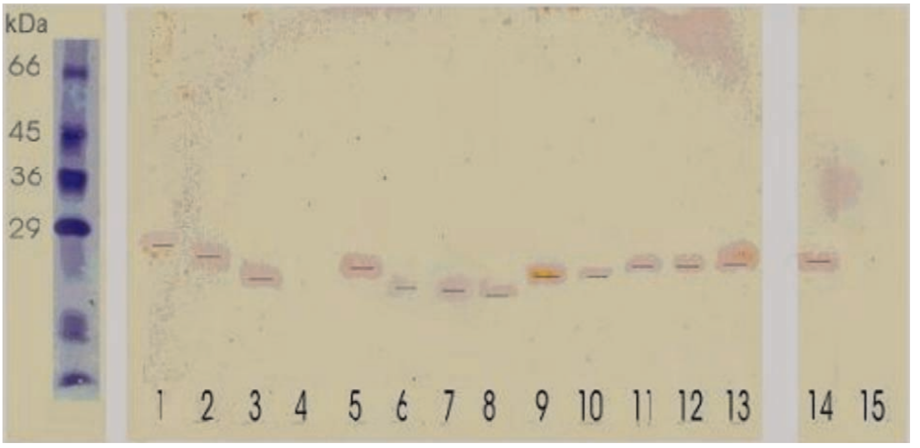
Results

The molecular weight of coat protein of two isolates: PNRSV-AprI/9 and PNRSV-PL21 could not be determined. Probably the virus concentration was too low in plant materials. The coat protein of all remaining 13 PNRSV isolates resolved as one major band by western blotting technique (Phot. 1). Estimates of Mγ varied among isolates within a narrow range of 24-25 kDa. Slightly different migrations were observed with coat proteins of the virus isolates tested, therefore these isolates were divided into three groups: group I – fast migrating isolates: PNRSV-PE56, PNRSV-Mk, PNRSV-PL1 and PNRSV-PL9, group II – slower migrating isolates: PNRSV-N2 and PNRSV-PL38 and group III – the slowest migrating isolates: PNRSV-AL1, PNRSV-AL17, PNRSV-SW2, PNRSV-PL7, PNRSV-R1, PNRSV-R2 and PNRSV-R3.

Table 1

List of PNRSV isolates studied

Isolate	Original host	Origin
PNRSV-AL1	Almond cv. 'Strout's Papershell'	Australia Dr. M. Wirthensohn (Adelaide University, Department of Horticulture, Viticulture and Oenology)
PNRSV-AL17	Almond cv. 'Tardy non Pareil'	Italy Dr. A. Myrta (Istituto Agronomio Mediterranea, Bari)
PNRSV-AprI/9	Apricot seedling (offspring of apricot cv. 'Schönborn Wyk')	Poland orchard, Ursynów (Warsaw Agricultural University, Department of Pomology), the second author collection
PNRSV-Mk	Sour cherry, unknown cultivar	Hungary Dr. M. Kölber (Crop Protection and Soil Conservation Service, Budapest)
PNRSV-N2	Sour cherry cv. 'Łutówka'	Poland orchard, Wilanów (Warsaw Agricultural University, Department of Pomology), the first author collection
PNRSV-PE56	Peach cv. 'Meredith'	Poland orchard, Lipowa near Sandomierz, the second author collection
PNRSV-PL1	Plum cv. 'Bluefre'	Poland orchard, Wilanów (Warsaw Agricultural University, Department of Pomology), the second author collection
PNRSV-PL7	Plum cv. 'Empress'	Poland orchard, Wilanów (Warsaw Agricultural University, Department of Pomology), the second author collection
PNRSV-PL9	Plum cv. 'Opal'	Poland orchard, Wilanów (Warsaw Agricultural University, Department of Pomology), the second author collection
PNRSV-PL21	Plum cv. 'Empress'	Poland orchard, Wilanów (Warsaw Agricultural University, Department of Pomology), the second author collection
PNRSV-PL38	Plum, unknown cultivar	Italy Dr. A. Myrta (Istituto Agronomio Mediterranea, Bari)
PNRSV-R1	Rose cv. 'Queen Elizabeth'	Poland rose collection (Warsaw Agricultural University, Department of Ornamental Plants), the second author collection
PNRSV-R2	Rose cv. 'Ingrid Bergman'	Poland Botanical Garden, Warsaw, the second author collection
PNRSV-R3	Rose cv. 'Montezuma'	Poland Botanical Garden, Warsaw, the second author collection
PNRSV-SW2	Sweet cherry cv. 'Jabonlay'	Poland orchard, Kutno, the second author collection



Phot. 1. Western blot analysis of PNRSV isolate; 1 – PNRSV-AL1, 2 – PNRSV-AL17, 3 – PNRSV-PE56, 4 – PNRSV-AprI/9, 5 – PNRSV-SW2, 6 – PNRSV-PL9, 7 – PNRSV-PL1, 8 – PNRSV-Mk, 9 – PNRSV-N2, 10 – PNRSV-PL38, 11 – PNRSV-R1, 12 – PNRSV-R2, 13 – PNRSV-R3, 14 – PNRSV-PL7, 15 – PNRSV-PL21
(photo by T. Malinowski)

Discussion

The molecular weights of coat protein of 13 PNRSV isolates studied, defined by western blotting technique, varied from 24 to 25 kDa. Barnett and Fulton (1969), Gonsalves and Fulton (1977), Ong (1987), Glasa et al. (2000) and Paduch-Cichal (2000) noted variation in Mγ among PNRSV isolates from 22 to 26 kDa. Results obtained by Crosslin and Mink (1992) also indicated to differences in coat protein Mγ (27 up to 29 kDa) among 22 PNRSV isolates. These virus isolates were divided into three electrophorotypes based on the relative distances of nucleoprotein migration in gel. One isolate, NRS-Hop, was assigned to elecrophorotype I. Electrophorotype II included isolates from peach, almond, sour cherry trees and rose plants, and electrophorotype III isolates – from peach, sweet and sour cherry trees. The 13 different PNRSV isolates studied here were also divided into three groups.

Crosslin and Mink (1992) suggested that there was not obvious relationship between biological properties of two PNRSV isolates (CH38 and CH39) and the coat protein Mγ. These isolates represented two major groups of biological variants of PNRSV commonly found in sweet cherry orchards in Washington. Isolate CH38 typified the group of isolates that induced rugose mosaic disease. Isolate CH39 represented isolates that remained symptomless in infected trees. Although these isolates differed in their biological properties and in their coat protein Mγ, they were serologically indistinguishable. The same phenomenon was noted in study with sour cherry PNRSV isolates described by Paduch-Cichal (2000) and Szyndel and Paduch-Cichal (1997). Our studies also have revealed some differences in host-range of the 13 PNRSV isolates, as well as in symptoms induced by these isolates on particular host plants (unpublished data). All PNRSV isolates presented in this paper could not be differentiated serologically either and appeared to belong to the CH-9 serotype, although some of them (AL1, PE56, Mk, N2) had probably common antigenic determinants with serotype CH-3 (Szyndel et al. 2006).

It has not been determined in the presented work, whether the observed variability in coat protein Mγ among PNRSV isolates results from differences in size, amino acid composition or structural configuration. Noel et al. (1979) reported that single amino acid substitution could alter the migration of a protein in polyacrylamide gels.

Streszczenie

RÓŻNICE W MOBILNOŚCI ELEKTROFORETYCZNEJ BIAŁKA KAPSYDU POMIĘDZY 13 IZOLATAMI WIRUSA NEKROTYCZNEJ PIERŚCIENIOWEJ PLAMISTOŚCI WIŚNI (PNRSV)

Porównano mobilność elektroforetyczną białka kapsydu 13 izolatów wirusa nekrotycznej pierścieniowej plamistości wiśni (*Prunus necrotic ringspot virus*, PNRSV) z użyciem techniki western blotting. Oszacowana pozorna masa cząsteczkowa białka kapsydu 13 izolatów PNRSV wynosiła od 24 do 25 kDa. Nie zdołano wyznaczyć masy cząsteczkowej białka kapsydu dwóch izolatów: PNRSV-AprI/9 i PNRSV-PL21.

Literature

Barnett O.W., Fulton R.W., 1969: Some chemical properties of Prunus necrotic ringspot and Tulare apple mosaic viruses. Virology 39: 556–561.

Crosslin J.M., Mink G.I., 1992: Biophysical differences among Prunus necrotic ringspot ilarvirus. Phytopathology 82: 200–206.

Glasa M., Subr Z., Myrta A., Kudela O., 2000: Detection and analysis of prunus necrotic ringspot virus isolates from Slovakia. Beitr. Züchtungsforsch. Bundesanst. Züchtungsforsch. Kulturpflanz. 6: 15–19.

Gonsalves D., Fulton R.W., 1977: Activation of prunus necrotic ringspot virus and rose mosaic virus by RNA4 components of some ilarviruses. Virology 81: 398–407.

Laemmli U.K., 1970: Cleavage of structural proteins during the assembly of the head bacteriophage T_4. Nature 227: 680–685.

Noel D., Nikaido K., Ames G.F.-L., 1979: A single amino acid substitution in a histidine-transport protein drastically alters its mobility in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Biochemistry 18: 4159–4165.

Ong C.-A., 1987: Separation and characterization of nucleoprotein components of prunus necrotic ringspot virus isolates. Typescript. Washington State University, Pullman.

Paduch-Cichal E., 2000: Wielostronna charakterystyka izolatów wirusa nekrotycznej pierścieniowej plamistości wiśni i wirusa karłowatości śliwy. Fundacja „Rozwój SGGW”, Warszawa.

Roossinck M.J., Bujarski J., Ding S.W., Hajimorad R., Hanada K., Scott S., Tousignant M., 2005: Family: Bromoviridae. In: Virus taxonomy. Classification and nomenclature of viruses. Eighth report of the International Committee on Taxonomy of Viruses. Eds. C.M. Fauquet, M.A. Mayo, J. Maniloff, U. Desselberger, L.A. Ball. Elsevier, San Diego: 1049–1059.

Sherwood J.L., 1987: Comparison of a filter paper immunobinding assay, western blotting and an enzyme linked immunosorbent assay for the detection of wheat streak mosaic virus. J. Phytopathol. 118: 68–75.

Sherwood J.L., Melouk H.A., 1986: A comparison of an enzyme linked immunosorbent assay (ELISA) and western blotting for detection of peanut mottle virus and peanut stripe virus. Science 13: 64–67.

Szyndel M.S., Paduch-Cichal E., 1997: Detection and serological comparison of PNRSV and PDV isolates by immunoelectron microscopy techniques. Phytopathol. Pol. 14: 19–27.

Szyndel M.S., Sala-Rejczak K., Paduch-Cichal E., 2006: Serological relationships among *Prunus necrotic ringspot virus* (PNRSV) isolates from stone fruit trees, rose and hop plants recognized by ISEM + decoration technique. Phytopathol. Pol. 40: 31–41.

Towbin H.T., Staehelin T., Gordon J., 1979: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. Procedure and some applications. Proc. Natl. Acad. Sci. USA 76: 4350–4354.

Authors' addresses:

Dr. hab. Elżbieta Paduch-Cichal, Kinga Sala-Rejczak M.Sc., Warsaw Agricultural University, Department of Plant Pathology, ul. Nowoursynowska 159, 02-787 Warsaw, Poland, e-mail: elzbieta_paduch_cichal@sggw.pl

Dr. Tadeusz Malinowski, Research Institute of Pomology and Floriculture, ul. Pomologiczna 18, 96-100 Skierniewice, Poland

Accepted for publication: 30.08.2007